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# Pheromonal and hormonal control of reproduction in the freshwater prawn, *Macrobrachium kistnensis*

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**Abstract.** In the freshwater prawn *Macrobrachium kistnensis* reared with males, the ovarian development occurred normally, whereas it was delayed in the absence of males. Testis and vas deferens extracts when added to the water in which females are being reared brought about normal development of ovary in comparison to those which were reared in boiled water or 'female water' alone. Therefore, mature males appear to be producing a pheromone which is necessary for the ovarian development of the female and the source of this might be vas deferens or testis. On the other hand, the central nervous tissue extracts stimulated vitellogenesis in oocytes. In particular, the brain extracts were more effective than those of thoracic ganglion in inducing this response. Thus, in the present study ovarian maturation and vitellogenesis appear to be under the pheromonal and hormonal controls.

**Keywords.** Pheromone; hormone; reproduction; *Macrobrachium kistnensis*.

## 1. Introduction

An endocrine mechanism for ovarian development in the prawn, *Leander serratus* was first reported by Panouse (1943, 1944, 1946) who found that eyestalk ablation induced rapid ovarian development in the nonbreeding season; selective surgical removal of the sinus gland from the eyestalk accelerated egg laying and implants of sinus glands into eyestalkless prawns prevented the otherwise anticipated ovarian development. Similar results have been reported for the crayfish, *Cambarus immunis*; the crabs, *Uca pugilator* (Brown and Jones 1947, 1949) and *Paratelphusa hydrodromous* (Anilkumar and Adiyodi 1980, 1985) and the shrimp, *Paratya compressa* (Takewaki and Yamamoto 1950).

The presence of an ovary stimulating factor in the brain and thoracic ganglion has been shown in several brachyuran species. These findings led to the current belief that, in crustaceans ovary growth is under dual hormonal control (Adiyodi and Adiyodi 1970; Adiyodi and Subramoniam 1983; Adiyodi 1985).

Ovarian development in the freshwater shrimp, *P. compressa* was delayed in the absence of the male. On the other hand if they are reared along with the males normal ovarian development occurred. Similar results were obtained when the females were reared with water containing the extracts of the testis or vas deferens (Takayanagi *et al* 1986).

These results show that mature male shrimps secrete an ovary stimulating pheromone. The effectiveness of the central nervous system and the pheromones produced by the male in stimulating ovarian growth of the freshwater prawn, *Macrobrachium kistnensis* has been presented in this paper.

## 2. Materials and methods

Animals used in these studies were kept in aquaria under a light cycle of L:D

14:10 at 25°C. Animals were fed on wheat floor and water was changed every 4 days; they were kept under these conditions for a week prior to use.

The ovaries were fixed with Bouin's solution, dehydrated by alcohol, and embedded in paraffin wax. Serial sections of 8 $\mu$m were stained with Harri's haematoxylin eosin.

Brain, thoracic ganglion and muscle removed from mature females was homogenized in glass distilled water for 15 min at 0°C. Each extract was centrifuged at 2000 rpm for 10 min and the supernatant (1 organ/animal) was added to 2 l of water.

The testis and vas deferens removed from mature males was homogenized as described above.

## 3. Results

When prawns were reared in the company of males, the ovary developed which is evident by the colour (yellowish to dark green), increased number of vitellogenic oocytes and increase in the diameter of the oocytes. When the prawns were reared in isolation, the ovarian growth was significantly low in comparison to those reared along with males (table 1).

When prawns were reared in the water in which males and females lived together, the ovary of the females showed greater development (colour changes from white to dark green, vitellogenic oocytes, and oocyte diameter increases) in comparison to those reared in isolated 'female water' and 'boiled water' (table 1).

Testis or vas deferens water significantly increased the ratio between previtellogenic, vitellogenic oocytes and oocyte diameter in comparison to the ovary of those reared in muscle water (table 1).

Oocytes of the ovary reared in thoracic ganglia or brain extracts were larger than those of controls (table 1), the brain extract being more effective than the extract of thoracic ganglion. Mean oocyte diameter was more than 320 $\mu$m with the ganglionic extracts compared with about 252 $\mu$m for the controls.

## 4. Discussion

The ovary of the freshwater prawn, *M. kistnensis* matured at a normal rate when reared along with the males, whereas in isolation it took a longer time to mature. Takayanagi *et al* (1986) reported similar results in the freshwater shrimp, *P. compressa*. Ovarian development also occurred normally in the prawns reared with water containing extracts of the testis or the vas deferens. Therefore, mature male prawns are shown to secrete an ovary stimulating pheromone and the sources may be some male organs such as the testis and the vas deferens. Similar findings were reported in the freshwater shrimp, *P. compressa* (Takayanagi *et al* 1986). On the other hand, the extracts of the central nervous system stimulated vitellogenesis in oocytes. In particular, the brain extracts were more effective than those of thoracic ganglion in inducing this response. These observations support the cytological studies of Matsumoto (1962) and Erribabu *et al* (1980) that the thoracic ganglion has an ovarian stimulating hormone. The results of Eastman-Reks and Fingerman (1984) on the crab, *U. pugilator* and Takayanagi *et al* (1986) on the shrimp, *P. compressa* support our findings, where an ovary stimulating hormone is

**Table 1.** Effect of various rearing conditions on ovarian development of *M. kistnensis* (rearing was done for 45 days).

| | Colour of ovary | | Ratio of previt: Vitell. oocytes | | Oocyte diameter $\mu m \pm SD$ | |
|---|---|---|---|---|---|---|
| Rearing conditions | Immature | Maturing | Immature | Maturing | Immature | Maturing |
| Rearing with male "male water" | Creamy | Dark green | 12:5 | 0:9 | 142·4 ± 3·6 | 287·3 ± 6·5 |
| Isolated rearing "female water" | Whitish | Faint green | 20:3 | 7:8 | 108·3 ± 5·6 | 201·6 ± 5·4 |
| Rearing in "male water" | Green | Dark green | 6:10 | 0:5 | 147·0 ± 6·8 | 328·4 ± 6·7 |
| Rearing in "female water" | Whitish | Faint green | 20:1 | 7:8 | 100·8 ± 5·5 | 192·8 ± 3·7 |
| Boiled "male water" | White | Creamy | 20:2 | 7:7 | 102·6 ± 4·7 | 196·6 ± 5·1 |
| Boiled "female water" | White | Creamy | 19:2 | 6:9 | 105·6 ± 4·3 | 210·6 ± 3·8 |
| Rearing in muscle extract | Creamy | Green | 15:3 | 2:9 | 131·2 ± 3·2 | 252·7 ± 5·2 |
| Rearing in testes-extract | Creamy | Green | 13:5 | 2:9 | 141·6 ± 4·1 | 260·5 ± 4·2 |
| Rearing in vas deferens extract | Creamy | Green | 12:6 | 1:9 | 150·2 ± 4·5 | 263·1 ± 4·3 |
| Rearing in thoracic ganglion extract | Light green | Green | 9:6 | 0:8 | 158·6 ± 5·3 | 298·4 ± 4·7 |
| Rearing in brain extract | Light green | Dark green | 6:7 | 0:6 | 168·6 ± 5·7 | 320·2 ± 5·9 |

shown to be present in the central nervous system and to act directly on ovarian vitellogenesis. The results of the present study indicate that the ovarian maturation, vitellogenesis in the freshwater prawn, *M. kistnensis* is under the control of pheromones produced by the male and hormones produced by the females.

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